

TNE

Page N . _____

12/95

Goal: To clone the TNE 35FY (mut)
into pUC99A.

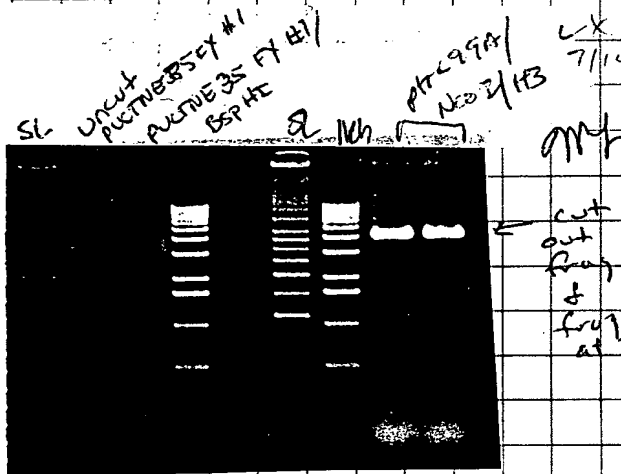
PUC TNE 35FY clone #1	30	pUC99A	5
10X R4	5	10X R2	2
H ₂ O	13	H ₂ O	11
BspHI	2	NcoI	1
	50 μ	H3	1

37°C - 1 hr.

Applied 5 μ l to
0.8% agarose gel
Gel run at 180V

20 μ l

Applied to
0.8% agarose
gel. Gel run
at 180V.



pUC99A/NcoI/H3 cut looks good
cut out 56 bp
4176
- 56
4120 bp

pUC TNE 35FY #1 / BspHI gives 1 kb, 1.3 kb,
+ 2.7 kb frag. Therefore, BspHI cuts
pUC TNE 35FY #1 3X. There must be
a BspHI in the insert.

13/95

EtOH ppt. Digest.
Dissolved in 20 μ l TE

BspHI
5 μ l
5 μ l 1X B.T.
10 μ l
Applied to 1 lane
of 0.8% agarose
gel. Gel run at 180V

BspHI
15 μ l DNA
2 μ l 1X R2
2 μ l H₂O
1 μ l H3 100/1
20 μ l
37°C - 1 hr.

To Page No. _____

Read & Understood by me,

Date

Invented by

Date

Lisha Xu

7/14/95

Recorded by

M. J. Long

7/13/95

TNE

ag N. _____

12/95

Goal: To clone the TNE 35FY (mut) into pUC99A.

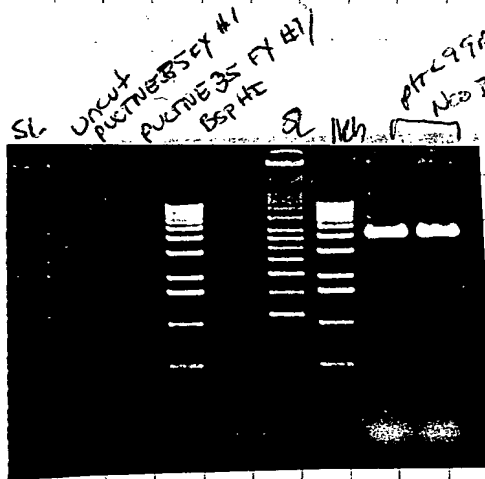
pUC TNE 35FY Clone #1	30	pUC99A	5
10xR4	5	10xR2	2
H ₂ O	13	H ₂ O	11
BspHI	2	NcoI	1
	50 μ	H3	1

37°C - 1hr.

Applied 5 μ l to 0.8% agarose gel
Gel run at 180V

20 μ l

Applied to 0.8% agarose gel
Gel run at 180V.



mt

cut out BspHI & fragment at 200C.

pUC99A/NcoI/H3 cut looks good
cut out 56 bp
4174
- 56
4120 bp

pUC99A/BspHI #1 / BspHI gives 1kb, 1.3kb, + 2.7kb frag. Therefore, BspHI cuts pUC99A/BspHI 3X. There must be a BspHI in the insert.

13/95

EtOH ppt. Digest.
Dissolved in 20 μ l TE

BspHI

BspHI

5 μ l
5 μ l NBT
10 μ l

15 μ l DNA
2 μ l 10xR2
2 μ l H₂O
1 μ l H3 100:1
20 μ l

Applied to 1 lane of 0.8% agarose gel
Gel run at 180V

37°C - 1hr.

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Date

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Date

Lizhu Xu

7/14/95

Recorded by

May Long

7/13/95

Project No. _____

Block No. _____

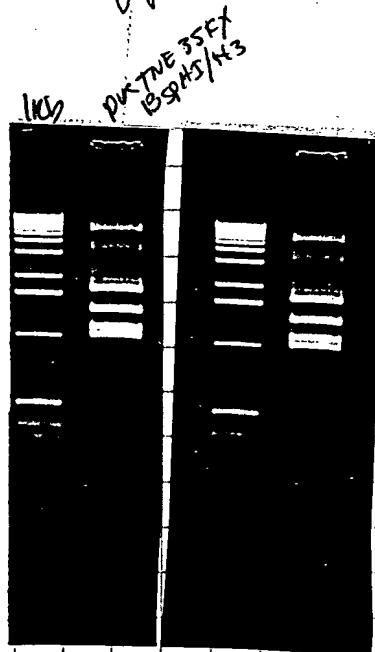
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TNE

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4/95

puc TNE 35F / BspH E → ETOH ppt. → Dissolved in 20 μ l 1x1
 buffer 2 μ l of H3 (10 μ l/l) was added 37°C - 1h
 applied to 1 lane of a 1% LMP agarose gel.
 Gel run at 180V.

7/14/95
GMYLX
7/20/95

cut the 200bp frag out +
 freeze at -20°C.

← 200bp frag

95

used the ~~phenol~~ ^{out} phenol extraction method to purify DNA.
 Dissolved in 10 μ l TE.

To Page No. _____

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Liz Xu

Date

7/20/95

Inv. nted by

Recorded by

Mary Longo

Date

7/18/95

TNE

Page No. _____

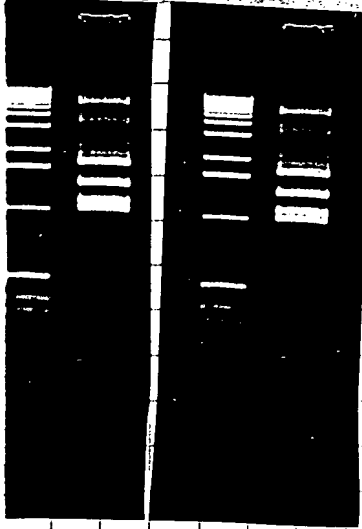
1/95

7/14/95
 Amy

puc TNE 35EX / BspH1E → ETOH ppt. → Dissolved in 20. μ l 1x.L
 buffer 2 μ l of H3 (100:1) was added 37°C - 1M
 applied to 1 lane of a 1% LMP agarose gel
 Gel run at 180V.

✓
 7/20/95

cut the 200bp frag out &
 freeze at -20°C.



← 200bp frag

35

used the ^{7/17/95} ~~phenol~~ phenol extraction method to purify DNA.
 Dissolved in 10 μ l TE.

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Read & Understood by m ,

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Liz Xu

7/20/95

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Mary Longo

7/18/95

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 Book N _____

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TNE

Page No. _____

Goal: To clone the TNE 35 Fy (mut) into pUC99A or a similar vector

New Scheme: pUC TNE 35 Fy (≈ 5.1 kb) → H3 → Klenow → SphI → SclI punct 2 kb frag

Clone into the SmaI/SphI site of pTTQ19.

pTTQ19	4	≈ 2 μg
10xR2	4	
H ₂ O	30	
SmaI	2	
100 μl	40	

pUC TNE 35 Fy	20	≈ 1 μg
10xR2	4	
H ₂ O	14	
H3	2	
100 μl	40 μl	

30°C - 1 hr.
 8/3/95

CMJ
 7/31/95

- 1 pUC TNE 35 Fy cut @ H3
- 2 pTTQ19 cut @ SmaI

cuts look good

pTTQ19/SmaI	40
SphI	2
100 μl	42

37°C - 1 hr.

pUC TNE 35 Fy/H3	40 μl
1xR2	10 μl
10 mM dNTP mix	2 μl
Klenow	0.5-1
	52-5

ice 5'
 EDTA to 20 mM
 phenol extracted
 TPEAgarose

ed & Understood by me,

ibhark

Date

8/3/95

Invented by

Recorded by

May Longo

Date

7/31/95

Project No. _____

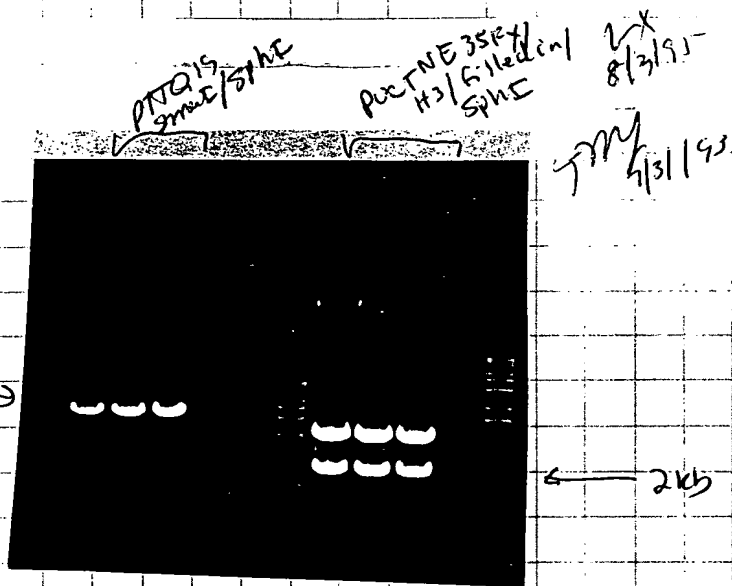
Book No. _____

TITLE

TNE

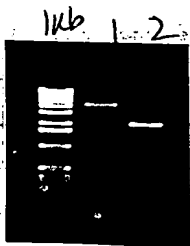
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pUC TNE 35S⁺ / H3 / filled in → resuspended in 40 μ l 1X RB
 2 μ l of 100 μ l SphI
 37°C - 1 hr.
 applied to a 0.9% agarose gel.
 Gel run at 180V.



cut bands out +
 freeze at -20°C

Gene clean the frag as usual.
 Dissolved in 10 μ l TE
 Applied 1 μ l to a 0.9% agarose gel.
 Gel run at 180V.



1 pTTA15 / smcI / SphI
 2 2 kb H3 / filled in / SphI frag
 from pUC TNE 35S⁺

~ 10 ng/ μ l = ...
 ~ 20 ng/ μ l = .

To Page 1

Witnessed & Understood by me,

Lidunyan

Date

8/8/95

Invented by

Recorded by

C. Man Loren

Date

8/1/95

Page No. _____

lig

TQ19 / SmaI / SphI 0.03 pmol/ μ l
 (b) H3 / Killed in / SphI 0.15 pmol/ μ l
 5X ligation buffer
 H₂O
 Ligase (10)

2
 1.5
 1
 4
 12.5
 1
 20.1

RT - 30 min.

Jason reform 2 μ l of the lig with 100 μ l DH10B CC.
 std reform. Plated 10% + 90% on yet amp plates. 37°C ON

#2 10% 90%
 18 ~150

picked 8 colonies into 3 ml of CG + amp 100. 37°C - ON.

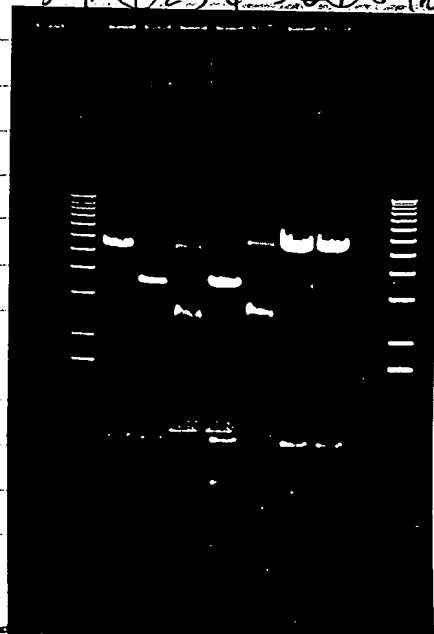
mp as usual. Dissolved in 5 μ l TE.

mp 3
 DCR4 2
 H₂O 13
 BphI 1
 EcoRI 1
 19

37°C - 1 hr.

Applied to a
 0.8% agarose
 gel. Gel
 run at 150V

sub PUCTNE 35 PY mut into
 SmaI / SphI site of pTQ19
 clones cut E SphI / EcoRI
 sl kb 1 2 3 4 5 6 7 8 9 10

ANY
PB/LS

To Page No. _____

sed & Understood by m ,

Date

8/3/95

Invented by

Date

2/13/95

Recorded by

Cory Long

Lisha Xu

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Book No. _____

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TNE

Page No. _____

lig

TQ19/SmaI/SphI 0.03 pmol/ul
kb H3/Cilled in SphI 0.15 pmol/ul
5X ligation buffer
H₂O
Ligase (10)

2
1.5
1
4
12.5
1
20 ul

RT - 30 min.

Jason reform 2 ul of the lig with 100 ul DH10B CC.
std reform Plated 10% + 90% on yet amp plates. 37°C ON

#2 10% 90%
18 ~150

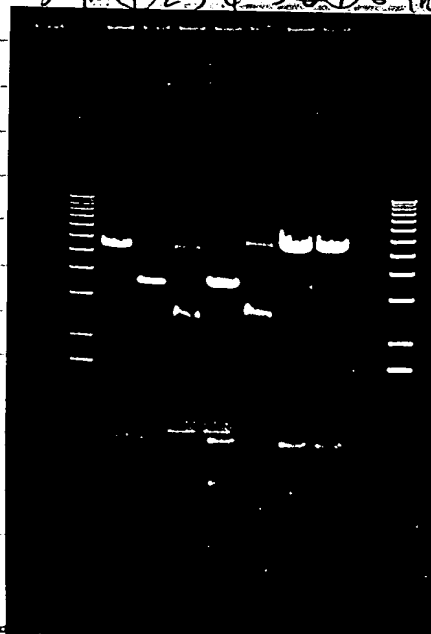
picked 8 colonies into 3 ml of CG + amp 100. 37°C - ON

mp as usual. Dissolved in 50 ul TE.

mp 3
DRL 2
H₂O 13
BpH 1
EcoRI 1
10

sub PUCTNE 35 BY mut into
SmaI/SphI site of pTQ19
cloned into SmaI/EcoRI
sl kb 1 2 3 4 5 6 7 8 9 10

ANY 8/1/95



37°C - 1 hr.
Applied to a
0.8% agarose
gel. Gel
run at 100V

4875
2000
6575

To Page No. _____

s d & Understood by me,

Lisha Xu

Dat

8/3/95

Inv nted by

R corded by

Colin Long

Date

8/13/95